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BRIEF COMMUNICATION

Comparative characteristics of growth and photosynthesis of sun and shade leaves from normal and pendulum walnut (*Juglans regia* L.) trees

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Abstract

Pendulum walnut leaves exhibited various adaptive responses related to the regulation of photon interception such as specific downward orientation, greater leaf area, and larger pigment pool. Changes in the regulation of PS2 such as higher thermal dissipation (NPQ) and lower quantum efficiency (Φ_{PS2}) that protect the photosynthetic apparatus against damages were also found. The growth and photosynthetic features of pendulum walnut leaf are interpreted as adaptations that allow the pendulum walnut tree to compensate the impaired ability by appropriate growth to ensure the energy needs for photosynthesis, respectively for biomass formation.

Additional key words: carotenoids; chlorophyll *a* fluorescence; growth habit; leaf dimensions; oxidative index; pigments.

Growth direction in woody plants depends on gravity and environmental stimuli. The weeping growth of the tree crown may arise when developmental and/or environmental controls of the gravitropic response are impaired (Myers *et al.* 1994, Hejnowicz 1997). Such trees are good models for understanding how plant organism, subjected to adverse environmental stimuli, undergoes appropriate physiological alterations that enable their survival.

Radiant energy is of great importance for plants with impaired gravity responses since their shoots may not reach sunlight. The decrease of shoot gravity response by growing on clinostat could lead to increased phototropic responses (Hangarter 1997). At least three different photon sensory systems are involved in modulating gravi- and phototropic development: phytochromes, which primarily absorb red and far-red radiation, and two blue radiation/UV-receptors (Chamovitz and Deng 1995, Quail *et al.* 1995). Carotenoids (Cars), mainly xanthophylls, function as photoreceptors in blue radiation responses (Quiñones and Zeiger 1994, Quinones *et al.* 1996).

The object of our study was the agroforestry species *Juglans regia* L. var. *pendulum*, which is characterised by agravitropic stems forming a weeping crown. The young branches of pendulum walnut initially grow upward then branches bend pointing with the tips straight down. There is enough evidence that amyloplasts in the sheath cells of pendulum walnut are lacking (Hejnowicz 1997). In pendulum walnut, a tree with impaired ability to seek and grow towards sunlight, we expected alterations in the leaf structure and function promoting the photosynthetic rate. Responses on leaf level such as changes in leaf orientation to the radiation, leaf area and thickness, chloroplast arrangement within the leaf, and changes in the pigment amount and composition bring about the regulation of photon interception (Björkman and Demmig-Adams 1994).

Leaves from sun (full sunlight) and shade (no more than 100 μmol m^{-2} s^{-1} photon flux density, PFD) sites of normal and pendula walnut trees were collected in June-July. The leaf length (L) and width (W), fresh and dry masses (FM, DM) were measured, leaf area (LA) and ratios leaf fresh/dry mass per leaf area (FM/LA; DM/LA) were calculated. Then leaf samples of 100-200 mg were ground and extracted with 80 % acetone. The absorbance

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Abbreviations: Cars – carotenoids; Chl – chlorophyll; F_0 – initial, F_v – variable, and F_m – maximum chlorophyll fluorescence, respectively; NPQ – non-photochemical Chl fluorescence quenching; PS – photosystem; Φ_{PS2} – apparent quantum efficiency.

of extracts was measured at 470 nm (Cars), 647 nm (chlorophyll *a*+*b*), and 664 nm (chlorophyll *a*) by a spectrophotometer *Shimadzu UV-200* and the content of pigments was calculated according to Lichtenthaler (1987). *In vivo* modulated Chl *a* prompt fluorescence was measured with a *PAM* fluorometer as described by Schreiber *et al.* (1986), at 25 °C after 1 h dark adaptation. F_0, the initial fluorescence yield in weak modulated radiation (0.075 μmol m^{-2} s^{-1} PFD), and F_m, maximum fluorescence yield during a saturating "white light" pulse (1 s at 3 000 μmol m^{-2} s^{-1} PFD by *Schott KL 1500* source) were determined on leaf discs (1 cm diameter). Quantum efficiency of photosystem 2 (PS2) was evaluated by F_v/F_m ratio. The quenching parameters were calculated as $NPQ = (F_m - F_m')/F_m'$ (Bilger and Björkman 1990) and $\Phi_{PS2} = (F_m' - F)/F_m'$ (Genty *et al.* 1989). Oxidative index was determined in sun and shade leaves subjected to 2-h photoinhibitory treatment (3 000 μmol m^{-2} s^{-1} PFD). Leaf samples (100-200 mg) were ground and extracted with chloroform : methanol (2 : 1), the extracts were evaporated under vacuum, the residues were solved in hexane, and A_{235nm}/A_{205nm} of aliquots of about 10 mg was determined (*Shimadzu UV-200*).

Pendula walnut leaves developed greater area and biomass (Table 1). There was an increase of L and FM of central rachis to which leaflets are formed in pairs; this part of the walnut leaf is mostly developed in sun pendula leaves. The longer rachis as well as the greater LA indicates increased elongating growth which could compensate the tree inability to move normally to the radiation source. The greater LA is a morphological feature positive for the light, as the photosynthetic surface increases. On the other hand, bigger LA in a tree crown of weeping form increases the shadiness, which can limit the photosynthesis and lead to switching on photoadaptive mechanisms. The enhanced leaf surface increases transpiration as well. So, specific downward orientation of sun leaves might be also a defence against the more significant loss of water. Both the FM/LA and DM/LA ratios were higher in sun leaves of both walnut trees indicating increased thickness as compared to the shade leaves (Table 1). The increased FM/LA ratio is associated with the adaptation to high irradiance but it may be a result of adjustment to changes of temperature and humidity as well (Boardman 1977). The increase in leaf thickness at high irradiance is related to increased photosynthetic efficiency. These ratios were lowest in shade pendula leaves and highest in sun pendula leaves (Table 1). It looks like that sun pendula leaves "work" most effectively in order to compensate low "work" capacity of shade pendula leaves.

Both the sun and shade leaves from pendula tree contained more Chl *a*+*b* than leaves from normal trees (Table 1). Leaves of plants grown at low irradiance contain more Chl due to the better grana formation of their chloroplasts than leaves grown at great irradiance (Anderson and Osmond 1987, Anderson 1999). Shade leaves of walnut trees of both growth types contained more Chl *b* (Table 1) and this increase is a typical feature associated with the better grana formation in the shade (Boardman 1977). Both sun and shade pendula leaves

Table 1. Growth indices (L – length; W – width; LA – leaf area; FM – fresh mass; DM – dry mass), contents of chlorophylls (Chl) and carotenoids (Cars), Chl *a* fluorescence parameters, and oxidative index (A_{235}/A_{205}) of normal and pendulum walnut leaves.

		Normal shade	Normal sun	Pendula shade	Pendula sun
L [cm]	leaflet	9.4±1.5	10.6±1.7	10.3±1.3	10.7±1.3
	rachis	16.2±0.9	17.4±3.5	19.3±1.2	18.6±2.9
W [cm]	leaflet	4.1±0.7	5.1±0.5	5.0±1.1	5.3±0.6
FM [g]	leaflet	0.6±0.2	0.9±0.2	0.7±0.1	1.1±0.3
	rachis	0.8±0.2	1.1±0.4	0.9±0.2	1.9±0.5
DM [% of FM]	leaflet	28.2	33.5	25.2	34.4
	rachis	23.0	25.3	21.0	27.2
LA [cm^2]	leaflet	19.3	27.0	25.8	28.4
FM/LA [g m^{-2}]	leaflet	331	333	271	387
DM/LA [g m^{-2}]	leaflet	88	111	68	133
Chl *a*+*b*		1.64±0.28	1.15±0.11	1.88±0.16	1.35±0.01
Chl *b* [g kg^{-1}(FM)]		0.40±0.02	0.31±0.02	0.50±0.03	0.35±0.02
Cars [g kg^{-1}(FM)]		0.28±0.01	0.23±0.01	0.33±0.02	0.40±0.04
Chl *a/b*		3.2	2.7	2.8	2.9
Cars/Chl		0.18	0.20	0.17	0.29
Leaflet Chl/FM [g kg^{-1}]		0.98	1.04	1.32	1.49
Leaflet Chl/LA [g m^{-2}]		0.50	0.40	0.51	0.52
F_v/F_m		0.81±0.01	0.83±0.01	0.79±0.01	0.83±0.01
NPQ		0.26±0.02	0.34±0.02	0.41±0.01	0.45±0.01
Φ_{PS2}		0.64±0.02	0.62±0.02	0.57±0.01	0.55±0.02
A_{235}/A_{205}		0.39±0.01	0.38±0.01	0.56±0.01	0.53±0.01

contained more Chl *b* than the leaves of normal walnut (Table 1). Chl *a/b* ratio was not significantly different, but the increase of Chl *b* content supports the shade type characteristic of pendula walnut leaves.

The content of Cars, evident also from the increased Cars/Chl ratio (Table 1), was the highest in the pendula leaves exposed to full sunlight. The formation of more Cars increases the absorption of photons and improves photoprotection. According to Demmig-Adams and Adams (1992) accumulation of Cars in response to high irradiance is related to the active operation of xanthophyll cycle. Also the results of Chl *a* fluorescence analysis, especially those characterising the non-photochemical quenching process (Table 1), indicated active function of xanthophyll cycle in pendula walnut leaves.

Sun leaves of both types of trees possessed similar quantum efficiency of PS2 (F_v/F_m), which was higher than F_v/F_m of shade leaves (Table 1). Thermal dissipation of excessive excitation energy (NPQ) was higher in the leaves of pendula tree. Φ_{PS2}, which indicates the apparent quantum efficiency of the electron transport, was lower in pendula leaves, probably due to increased thermal dissipation and decreased photochemistry.

Photosynthesis generates numerous highly reactive molecules that cause photooxidative damage to the photosynthetic apparatus (Foyer *et al.* 1994, Niyogi 1999). These oxidative damages can be a serious reason for the increased photosensitivity of the leaves (Foyer *et al.* 1994). PS2 is most sensitive to such a damage, which may decrease the efficiency and/or maximum rate of photosynthesis (Niyogi 1999). The oxidative index (A_{235}/A_{205}) calculated for comparing the damages in sun and shade leaves subjected to photoinhibitory treatment was higher in pendula leaves what indicated more severe oxidative damage (Table 1).

NPQ and Φ_{PS2} differences in the leaves of pendula tree and the enhancement of A_{235}/A_{205} ratio can be consequence from differences in thylakoid membrane structure and/or result of more unfavourable environment for pendula walnut tree. The greater capacity to dissipate excess excitation energy thermally (NPQ) is related to an increase in the xanthophyll cycle pool size, especially that of zeaxanthin content (Demmig-Adams and Adams 1992). As mentioned above, higher content of Cars (Table 1), even though indirectly, supports such assumption. Besides photoprotective and chloroplast membrane stabilising functions (Demmig-Adams and Adams 1992, Niyogi 1999) pigments of xanthophyll cycle function in the control of light dependent phototropic and gravitropic movements. Zeaxanthin or xantophyll turnover is crucial link in signal transduction pathway producing chloroplast and stomatal movements and shoot curvatures in light (Zeiger and Zhu 1998, Tlalka *et al.* 1999, Talbott *et al.* 2002). These pigments as well as the other chromophors may modulate hormonal balance (auxin, abscisic acid, gibberellin, cytokinin) that provokes definite growth and developmental responses to environmental and gravity stimuli (Hangarter 1997, Krapiel and Miginiac 1997). The leaves of pendula walnut contain more Cars which may be a physiological need developed to compensate the missing ability of the tree to establish such an architecture that optimises the photon interception.

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